# Distribution of Thioredoxins in Cyanobacteria

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The presence of thioredoxin was demonstrated in 20 strains of cyanobacteria as well as in one phototrophic bacterium *Rhodopseudomonas sulfidophila* and in *Thiobacillus denitrificans*. Thioredoxin activity was not found in *Cyanophora paradoxa* and in *Porphyridium cruentum* using the thioredoxin-dependent PAPS-sulfotransferase activity from *Synechococcus* 6301 as assay system.

## Introduction

Thioredoxins have been found originally as a cofactor for ribonucleotid reduction in bacteria [1, 2]. Recent developments demonstrated the presence of thioredoxins in one cyanobacterium (*Synechococcus*) [3], one green alga (*Scenedesmus*) [4] and in higher plants [5, 6]. It was further established that in spinach thioredoxins are needed for activation of certain enzymes needed in carbohydrate metabolism including the fructose bisphosphatase of spinach [6, 7]. We found recently that the sulfotransferase of *Synechococcus* 6301 is activated by thioredoxin [2] and it was shown that thioredoxins from different sources could be used for activation with this cyanobacterial sulfotransferase [8], and we have shown further that thioredoxin activates the fructose-bisphosphatase from *Synechococcus* 6301 [9]. Thus, it is evident that thioredoxins are regulatory proteins, which might govern cellular metabolism, especially if fluctuations of enzyme activities are observed. Since we had found and isolated a thioredoxin from the cyanobacterium *Synechococcus* 6301, we wanted to get more information about the distribution of thioredoxins in this group of organisms.

## Materials and Methods

a) *Biological materials:* All cyanobacteria including *Cyanophora paradoxa* were a generous gift of Prof. Dr. Stanier, Institute Pasteur, Paris. *Anabaena variabilis* was obtained from Dr. Schilling, Botanisches Institut, University of München (strain of C. P. Wolk); *Thiobacillus denitrificans* and *Rhodopseudomonas sulfidophila* were obtained from Prof. Dr. Trüper, Institut für Mikrobiologie, University of Bonn; and *Porphyridium cruentum* was obtained from Dr. Köst, Botanisches Institut, University of München. Purified thioredoxin from *E. coli* was a gift of Prof. Dr. Follmann, Fachbereich Chemie, University of Marburg. *Synechococcus* 6301 was grown as acenic culture in the BG-11 medium [10].

b) *Assay systems and analytical procedures:* The preparation of PAPS-sulfotransferase from *Synechococcus 6301*, the isolation of thioredoxin from the same organism, the measurement of the PAPS-sulfotransferase, the preparation of PAPS and other details of interest are described in an earlier paper [3]. Samples to be analyzed for thioredoxin were prepared in the following way: 2 g of material (wet weight) was mixed with 4 ml of 0.02 M Tris-HCl-buffer pH 8.0 and broken in a french press at 12,000 psi. After centrifugation for 15 min at 12,000 × *g* the supernatant was heated for 5 min in a boiling water bath. After cooling the extract was centrifuged again. 0.2 ml of this supernatant was used for the measurement of thioredoxin activity. Each assay contained (in μmol): Tris-HCl pH 8.0: 100; $MgCl_2$: 10; $CaCl_2$: 2; dithioerythritol: 10; PAPS: 0.05 (spec. activity, 820 cpm/nmol); 280 μg of *Synechococcus* sulfotransferase and 0.2 ml of the sample to be analyzed in a total volume of 1 ml. The mixtures were incubated for 1 hour under $N_2$. After addition of carriersulfite the reaction was stopped by acidification with HCl and the $SO_2$ liberated was trapped in 1 M triethanolamin, and the radioactivity determined as described [3].

## Results and Discussion

It was shown recently by us that the cyanobacterium *Synechococcus* 6301 contains a PAPS-dependent sulfotransferase, which needed a heat-stable protein as cofactor. This heat-stable protein was identified as a thioredoxin [3, 8] and we were able to demonstrate that this cyanobacterial sulfotransferase will accept thioredoxins from a variety of sources including *E. coli, Scenedesmus,* and spinach [8]. This shows, that the sulfotransferase can be used as analytical tool for thioredoxin assays. For the measurement of thioredoxins in different cyanobacteria we have made use from our finding that the *Synechococcus* thioredoxin is heat-stable; therefore all sam-

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Analysis for thioredoxin in different strains of cyanobacteria using the thioredoxin-dependent sulfotransferase of Synechococcus 6301.

| | nmol acid-volatile radioactivity formed | % of control |
|---|---|---|
| Transferase *Synechococcus* 6301 | | |
| + *Anabaena variabilis* (grown on $NO_3^-$) | | |
| + *Anabaena variabilis* (grown on $N_2$) | | |
| + *Anabaena* 7120 | | |
| + *Anabaena* 6411 | | |
| + *Calothrix* 6303 | 0.41 | 89 |
| + *Chroococcidiopsis* 7203 | = | |
| + *Cyanophora paradoxa* | 0.46 | 100 |
| + *Fischerella* 7115 | 0.86 | 188 |
| + *Gloeothece* 6501 | 0.46 | 100 |
| + *Gloeothece* 7109 | 0.37 | 80 |
| + LPP 6306 | 1.03 | 225 |
| + LPP 6402 | 0.75 | 164 |
| + *Nostoc* 6310 | 1.15 | 251 |
| + *Nostoc* 6720 | 0.87 | 190 |
| + *Oscillatoria* 7101 | 1.06 | 231 |
| + *Plectonema* 73110 | 1.36 | 296 |
| + *Spirulina* 6313 | 0.67 | 146 |
| + *Synechococcus* 6301 | 3.26 | 720 |
| + *Synechococcus* 6907 | 3.50 | 762 |
| + *Synechococcus* 6312 | 2.08 | 450 |
| + *Synechococcus* 7418 | 1.58 | 340 |
| + *Synechocystis* 6701 | 2.13 | 462 |
| + *Thiobacillus denitrificans* | 3.30 | 718 |
| + *Rhodopseudomonas sulfidophila* W 4 | 1.26 | 274 |
| + *Porphyridium cruentum* | 0.46 | 100 |
| + *Spinacia oleracea* | 2.60 | 567 |
| + purified thioredoxin from *E. coli* | 7.73 | 1680 |
| + purified thioredoxin from *Synechococcus* | 7.80 | 1714 |

=, has a heat-stable sulfotransferase and can therefore not be determined in this assay system.

ples to be analyzed for thioredoxin were heated prior to the measurement. Controls were run to ensure that all sulfotransferase activities were inactivated by the heat treatment. With this assay system thioredoxin was detected in most strains analyzed. The data of the Table show that the activation of the sulfotransferase is best in the coccid forms of the *Synechococcus-Synechocystis* group, whereas little activity was found in *Gloeothece* and *Calothrix*. No activity was found in *Cyanophora paradoxa* using the PAPS-sulfotransferase system of *Synechococcus*. However, we were able to demonstrate that in *Cyanophora* a heat-stable substance is present which activates in the presence of mono- or dithiols the Cyanophora-PAPS-sulfotransferase, suggesting that in this alga another type of activation-protein is present [11].

The table has listed *Chroococcidiopsis* with no data. This is due to the fact that Chroococcidiopsis contains a 3′-phosphatase and a APS-sulfotransferase, which are heat-stable and which interfere with the assay system used. However, after separation of the APS-sulfotransferase and thioredoxin on a Sephadex G-100 column we can demonstrate that thioredoxin is present and that Chroococcidiopsis contains a thioredoxin-dependent APS-Sulfotransferase [11, 12].

The data of the table show, that *Anabena variabilis* changes the thioredoxin content when grown in different ways. If grown in the BG-11 medium with nitrate as nitrogen source this alga forms no heterocysts and the amount of thioredoxin detected is low. When cells were grown in Allen's medium [13] using $N_2$ as nitrogen source, heterocysts are formed. It is evident that under conditions of heterocyst formation more thioredoxin is found than in the controls grown on nitrate. This suggests that thioredoxin might have some function in the nitrogen flow from $N_2$ to protein. This should be seen in connection with the recent observation that the glutamin synthetase of this alga is enhanced by light [14].

Our data demonstrate that thioredoxins are present in a variety of cyanobacterial strains. This suggests that thioredoxins might function in this group of organisms in the regulation of the sulfur flow [3], in the regulation of the carbon flow [9, 15] and possibly in the regulation of the nitrogen flow.

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